<110> Lehmann, Juergen Michael
      Shiau, Andrew Kwan-Nan
      Tularik Inc.

<120> CAR Modulators: Screening and Treatment of
      Hypercholesterolemia

<130> 018781-004110US

<140> US 09/760,364
<141> 2001-01-12

<150> US 60/176,398
<151> 2000-01-13

<160> 14

<170> PatentIn Ver. 2.1

<210> 1
<211> 348
<212> PRT
<213> Homo sapiens

<220>
<223> human constitutive androstane receptor (CAR) alpha
      (hCARa)

<400> 1
Met Ala Ser Arg Glu Asp Glu Leu Arg Asn Cys Val Val Cys Gly Asp
 1   5   10   15

Gln Ala Thr Gly Tyr His Phe Asn Ala Leu Thr Cys Glu Gly Cys Lys
            20   25   30

Gly Phe Phe Arg Arg Thr Val Ser Lys Ser Ile Gly Pro Thr Cys Pro
        35   40   45

Phe Ala Gly Ser Cys Glu Val Ser Lys Thr Gln Arg Arg His Cys Pro
    50   55   60

Ala Cys Arg Leu Gln Lys Cys Leu Asp Ala Gly Met Arg Lys Asp Met
65   70   75   80

Ile Leu Ser Ala Glu Ala Leu Ala Leu Arg Arg Ala Lys Gln Ala Gln
                85   90   95

Arg Arg Ala Gln Gln Thr Pro Val Gln Leu Ser Lys Glu Gln Glu Glu
            100   105   110

Leu Ile Arg Thr Leu Leu Gly Ala His Thr Arg His Met Gly Thr Met
            115   120   125

Phe Glu Gln Phe Val Gln Phe Arg Pro Pro Ala His Leu Phe Ile His
    130   135   140

His Gln Pro Leu Pro Thr Leu Ala Pro Val Leu Pro Leu Val Thr His
145   150   155   160

1

Phe Ala Asp Ile Asn Thr Phe Met Val Leu Gln Val Ile Lys Phe Thr
                165   170   175

Lys Asp Leu Pro Val Phe Arg Ser Leu Pro Ile Glu Asp Gln Ile Ser
            180   185   190

Leu Leu Lys Gly Ala Ala Val Glu Ile Cys His Ile Val Leu Asn Thr
            195   200   205

Thr Phe Cys Leu Gln Thr Gln Asn Phe Leu Cys Gly Pro Leu Arg Tyr
    210   215   220

Thr Ile Glu Asp Gly Ala Arg Val Gly Phe Gln Val Glu Phe Leu Glu
225   230   235   240

Leu Leu Phe His Phe His Gly Thr Leu Arg Lys Leu Gln Leu Gln Glu
            245   250   255

Pro Glu Tyr Val Leu Leu Ala Ala Met Ala Leu Phe Ser Pro Asp Arg
            260   265   270

Pro Gly Val Thr Gln Arg Asp Glu Ile Asp Gln Leu Gln Glu Glu Met
            275   280   285

Ala Leu Thr Leu Gln Ser Tyr Ile Lys Gly Gln Gln Arg Arg Pro Arg
    290   295   300

Asp Arg Phe Leu Tyr Ala Lys Leu Leu Gly Leu Leu Ala Glu Leu Arg
305   310   315   320

Ser Ile Asn Glu Ala Tyr Gly Tyr Gln Ile Gln His Ile Gln Gly Leu
            325   330   335

Ser Ala Met Met Pro Leu Leu Gln Glu Ile Cys Ser
            340   345

<210> 2
<211> 358
<212> PRT
<213> Mus musculus

<220>
<223> mouse constitutive androstane receptor (CAR) beta 1
      (mCARbeta1, mCAR1)

<400> 2
Met Thr Ala Met Leu Thr Leu Glu Thr Met Ala Ser Glu Glu Glu Tyr
1   5   10   15

Gly Pro Arg Asn Cys Val Val Cys Gly Asp Arg Ala Thr Gly Tyr His
            20   25   30

Phe His Ala Leu Thr Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Thr
            35   40   45

Val Ser Lys Thr Ile Gly Pro Ile Cys Pro Phe Ala Gly Arg Cys Glu
    50   55   60

Val Ser Lys Ala Gln Arg Arg His Cys Pro Ala Cys Arg Leu Gln Lys
65   70   75   80

2

Cys Leu Asn Val Gly Met Arg Lys Asp Met Ile Leu Ser Ala Glu Ala
                    85   90   95

Leu Ala Leu Arg Arg Ala Arg Gln Ala Gln Arg Arg Ala Glu Lys Ala
                100   105   110

Ser Leu Gln Leu Asn Gln Gln Gln Lys Glu Leu Val Gln Ile Leu Leu
            115   120   125

Gly Ala His Thr Arg His Val Gly Pro Leu Phe Asp Gln Phe Val Gln
        130   135   140

Phe Lys Pro Pro Ala Tyr Leu Phe Met His His Arg Pro Phe Gln Pro
145   150   155   160

Arg Gly Pro Val Leu Pro Leu Leu Thr His Phe Ala Asp Ile Asn Thr
                165   170   175

Phe Met Val Gln Gln Ile Ile Lys Phe Thr Lys Asp Leu Pro Leu Phe
                180   185   190

Arg Ser Leu Thr Met Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala Ala
            195   200   205

Val Glu Ile Leu His Ile Ser Leu Asn Thr Thr Phe Cys Leu Gln Thr
210   215   220

Glu Asn Phe Phe Cys Gly Pro Leu Cys Tyr Lys Met Glu Asp Ala Val
225   230   235   240

His Ala Gly Phe Gln Tyr Glu Phe Leu Glu Ser Ile Leu His Phe His
                245   250   255

Lys Asn Leu Lys Gly Leu His Leu Gln Glu Pro Glu Tyr Val Leu Met
                260   265   270

Ala Ala Thr Ala Leu Phe Ser Pro Asp Arg Pro Gly Val Thr Gln Arg
                275   280   285

Glu Glu Ile Asp Gln Leu Gln Glu Glu Met Ala Leu Ile Leu Asn Asn
290   295   300

His Ile Met Glu Gln Gln Ser Arg Leu Gln Ser Arg Phe Leu Tyr Ala
305   310   315   320

Lys Leu Met Gly Leu Leu Ala Asp Leu Arg Ser Ile Asn Asn Ala Tyr
                325   330   335

Ser Tyr Glu Leu Gln Arg Leu Glu Glu Leu Ser Ala Met Thr Pro Leu
                340   345   350

Leu Gly Glu Ile Cys Ser
        355

<210> 3
<211> 286
<212> PRT
<213> Mus musculus

3

<220>
<223> mouse constitutive androstane receptor (CAR) beta 2
      (mCARbeta2, mCAR2)

<400> 3
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 1               5                  10                      15

Gly Pro Arg Asn Cys Val Val Cys Gly Asp Arg Ala Thr Gly Tyr His
            20                  25                  30

Phe His Ala Leu Thr Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Thr
            35              40                  45

Val Ser Lys Thr Ile Gly Pro Ile Cys Pro Phe Ala Gly Arg Cys Glu
        50                  55              60

Val Ser Lys Ala Gln Arg Arg His Cys Pro Ala Cys Arg Leu Gln Lys
65              70                  75                      80

Cys Leu Asn Val Gly Met Arg Lys Asp Met Ile Leu Ser Ala Glu Ala
                85                  90                  95

Leu Ala Leu Arg Arg Ala Arg Gln Ala Gln Arg Arg Ala Glu Lys Ala
            100             105                 110

Ser Leu Gln Leu Asn Gln Gln Gln Lys Glu Leu Val Gln Ile Leu Leu
            115             120                 125

Gly Ala His Thr Arg His Val Gly Pro Leu Phe Asp Gln Phe Val Gln
        130             135                 140

Phe Lys Pro Pro Ala Tyr Leu Phe Met His His Arg Pro Phe Gln Pro
145                 150                 155                 160

Arg Gly Pro Val Leu Pro Leu Leu Thr His Phe Ala Asp Ile Asn Thr
                165                 170                 175

Phe Met Val Gln Gln Ile Ile Lys Phe Thr Lys Asp Leu Pro Leu Phe
                180                 185                 190

Arg Ser Leu Thr Met Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala Ala
            195                 200                 205

Val Glu Ile Leu His Ile Ser Leu Asn Thr Thr Phe Cys Leu Gln Thr
        210                 215                 220

Glu Asn Phe Phe Cys Gly Pro Leu Cys Tyr Lys Met Glu Asp Ala Val
225                 230                 235                 240

His Ala Gly Phe Gln Tyr Glu Phe Leu Glu Ser Ile Leu His Phe His
                245                 250                 255

Lys Asn Leu Lys Gly Leu His Leu Gln Glu Pro Glu Tyr Val Leu Met
                260                 265                 270

Ala Ala Thr Ala Leu Phe Ser Pro Gly Phe Cys Met Gln Ser
                275             280                 285
```

4

```
<210> 4
<211> 492
<212> DNA
<213> Mus musculus

<220>
<223> murine CARbeta genomic sequence - Section A,
      portion of CARbeta intron sequence in left arm of
      targeting construct

<220>
<221> modified_base
<222> (74)
<223> n = g, a, c or t

<400> 4
aaaatttacc caacatagat ttatctaatg taattcctat ctgcagaaca tccaaatact 60
ttggaaatta tttnttgtgg ttgtagctgt ttgaatgtaa acatatattc aaaaaaactc 120
ttcatggtga tgtagcattg ggcaagctat gaggatacct acttctggtt atttactaaa 180
agttgatagc caggcagtgg tggcacacac ctttaatccc agcacttggg aggcagaggc 240
aggtggaatt atgagtttga ggccagcctg gtctacagag tgggttcaag gtcagccagg 300
gctacacaga gaaaccctgt ctcaaaaaga aggaggagga ggaggaaaga ggaagaggag 360
gaagaagatc ttttgttttg agatagcata cagtgaaaat ttcggtttct ttagcaactc 420
agttgtgtca catgatgtct ttctggaagc tgtcttgtga gcagacatgt gatgtttatc 480
acaatagaaa gc                                                   492


<210> 5
<211> 1779
<212> DNA
<213> Mus musculus

<220>
<223> murine CARbeta genomic sequence - Section B,
      portion of CARbeta genomic sequence 5' to Section A

<400> 5
aaagaggtca tcaggcttgg cagcaagtgc ctttgcctac cgagtcttta caccagctcc 60
accgtggttt ttgagacagt ctcccactgg actggatttc agcaagaaag ctaggcttgc 120
cttcttgtct ctgcctcctt ggcattggaa ttatgagttg ttccaccgtg ccattttaa 180
aaatgtaggt tctaggaatt aaactcggct ctcggtgctt atatagtgag tactttacag 240
agggagtcac cttgccagca cctagaattc actttattc atatcccagt ctccccacgt 300
aagaaagtgg gatcccttct agtgttacac ctaagttctt agttggatac cgaagtcttt 360
ttttaacag atctctgggg ctcagaaggc aagagctcct tgcagaggat ttaacctcaa 420
ttcctagtac tcaacttgcc agctcataac tgcctataac tctagtccca gaagatcaga 480
cattgtcctc tgatctctgt gggtactagg tatatacatt taaaaaaaat caataaaaaa 540
tttaaaaaaa gaaaagaaaa agaaagaaag aaatcctttt gggagcctgg tataattgtt 600
atagctacct ttttttttttt ttttttttttt tttttacca tttgcaaact gcacgtgaaa 660
aagcttgcca tctctcccat tgtttcctgg cttattcagg atccatgcaa aaaggggagt 720
gtagatttag cctaaagctc acccacaggg aaatcctcca ggagtctagt aagcagcagc 780
ttttaatgag tcatgaggtc ctggcccctc cccatctgcc accaaccaac acttctcggg 840
catgctagga accccaccc caccccacac ccacacccag gtctttgccc tgggtccaga 900
gtctgggtcc tacctacata tggcaccgag gatacctaga ggcccatgc aagagaaggc 960
ccttgttttc caggcactaa ggaccgcagt ccctaattcc tggcagttcc tgagatctca 1020
aggaaagcag ggtcagcgag gaggcctggg gagaggaggc atcctacacc cgatcttgtg 1080
gcctgctgcc taagggaaac aggtaggtaa tccgttggag gccagagaca aaaagcaaca 1140
ttttttgcttt taatgtcctc agtgctgggg agccggtgt caggctgggc agtcttggga 1200
agagattctg tagaggagag agaagagagt cctatggccc agtgctgatt ctcaactcct 1260
cccacattca ggagaccatg acagctatgc taacactaga aaccatggcc agtgaagaag 1320
aatatgggcc gaggaactgt gtggtgtgtg gagaccgggc cacaggctat catttccacg 1380
ccctgacttg tgagggctgc aagggcttct tcaggtgaat gcttcctccc caacagaaac 1440
```

```
aaccccgaca tttctatcag tccacctttta aacactggta cacctccaag ttataatcct 1500
cttgcagcta agctgcactg cccagtgtct agcactctca atcttgctga ccacaacgca 1560
gtgtgaaact ggtgacctaa tgacaaggca ggttaaccat ttgtcccaga gacagagcct 1620
aagagtcaag aacacttgtg tagcacacac tacctgcaaa gcaccgagat gattgccaca 1680
cgagggttcc tgagtaacct tgtgttctca tgaaaacgct ccaactacct ctgaagacct 1740
ttgagcacag ctcagatgag tctgttgtta aatcgatcc                        1779
```

<210> 6
<211> 485
<212> DNA
<213> Mus musculus

<220>
<223> murine CARbeta genomic sequence - Section C,
      CARbeta intron sequence in right arm of targeting
      construct

<400> 6
```
tgcattgctt tctactgaag tgtatcacag atgaatatga gatcgacaga aagtgtgcag 60
ggatcccct gccatctgga aacacttaat tcaatgaagt cccaaggaag cctcagaaac 120
tctttcttcc ttcctccttc cttatctggg gaggtggagt ggccccaact gaagggatgg 180
ctgaaaggtg ctcgctgctg ttctcaacag ctttgtcatc tctcttgcct gacacagtga 240
tactgtcagc agaagccctg gcattgcggc gagccagaca ggcacagcgg cgggcagaga 300
aagcatcttt gcaactgaat cagcagcaga aagaactggt ccagatcctc ctcggggccc 360
acactcgcca tgtgggcccc atgtttgacc agtttgtgca gttcaaggtg agaacttaac 420
caggatgtga cctgggtacc tgaggaggta acccacagaa gaaggctatg ccctgatgga 480
ggaca                                                             485
```

<210> 7
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:sensor peptide,
      rhodamine-labeled peptide derived from the
      receptor binding domain of coactivator SRC-1

<400> 7
```
Ile Leu Arg Lys Leu Leu Gln Glu
  1               5
```

<210> 8
<211> 1390
<212> DNA
<213> Mesocricetus auratus

<220>
<223> hamster constitutive androstane receptor (CAR)

<220>
<221> modified_base
<222> (1)..(1390)
<223> n = g, a, c or t

<400> 8
```
cttgttttct agggaccaag gacaatccct aattcctgca gttcctgaga ccacaaggaa 60
agcagggtca tcgtggaggc ttggagacag gcatctcata ccagattttg tgacctgcgt 120
```

```
gtgtcatact gcctaagaga aacaggagac catgacagct acgctaacac tcgaaaccaa 180
ggccagtgga gaggaatatg gaccgaggaa ctgtgtggtg tgtggagacc gagccacggg 240
ctaccatttc catgccctga cttgtgaggg ctgcaaaggc ttcttcagac gaactgtcag 300
caaaaccatt agtcccatct gtccatttc tggaagctgt gagatcagca gagcccagag 360
acgccactgc ccagcctgca ggttgcagaa gtgcctaaac gctggcatga ggaaagacat 420
gatactgtca gcagaagccc tgtcgttgcg gcgagccagg caggcacagc ggcgggcaca 480
aaaagcttcc gtgcagatga ctcaggagcg gaaggagctg gtccagaccc tcatagggggc 540
ccacacccgc cacatgggcc ccatgtttga ccagtttgtg aagctcaggc ctccagctta 600
cctgttcacc catcaccggc cctcctcccc gctggtcccc cccgcgttac cactgctcac 660
acactttgca gatgtcaaca ctttcatggt gcagcagatt atcaagttca ccaaggaact 720
gcccctttttt cggtccctac ccgtggagga ccagatctcc cttctcaagg gagcagctgt 780
ggaaatattg catatctcac tcaacactac tttctgtctt caaacacaga atttcttctg 840
tgggccactt tgctacaaaa tggaagacgc agcccacgca gggttccggt acgaatatgt 900
ggagttgatc tttcgcttcc atgggacact gaagcgactg cagctggaatca 960
tgtgctcatg actgccatgg ccctcttctc tcctgacagg cctggaatca cccagagaga 1020
agagattgac cagctgcaag aggagatggc actgatttttg aacaactaca ttatggaaca 1080
gcagccaagg ccccagagtc ggtttctgta cgcaaagctg atgggcctgc tggctgagct 1140
ccggagcata aacaatgcat actcatatga aatacggcgc atccagggac tgtccgctat 1200
gatgccacta cttgggggaaa tctgcagctg aggctcaggc ttgcctcctt ccccagggcc 1260
cctgggattc attggactgg aaaggggaaa ttgctgagct aaaaggagct cagtgacagc 1320
aaaaaacact ggacagtngg aaaaaaannn nnnnnnnnnnn aaaagcgacc tgcccgggcg 1380
gccgttcagc                                                      1390
```


<210> 9
<211> 359
<212> PRT
<213> Mesocricetus auratus

<220>
<223> predicted amino acid sequence of hamster
      constitutive androstane receptor (CAR)

<400> 9
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Met Thr Ala Thr Leu Thr Leu Glu Thr Lys Ala Ser Gly Glu Glu Tyr
 1               5                  10                  15

Gly Pro Arg Asn Cys Val Val Cys Gly Asp Arg Ala Thr Gly Tyr His
                20                  25                  30

Phe His Ala Leu Thr Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Thr
                35                  40                  45

Val Ser Lys Thr Ile Ser Pro Ile Cys Pro Phe Ser Gly Ser Cys Glu
    50                  55                  60

Ile Ser Arg Ala Gln Arg Arg His Cys Pro Ala Cys Arg Leu Gln Lys
65                  70                  75                  80

Cys Leu Asn Ala Gly Met Arg Lys Asp Met Ile Leu Ser Ala Glu Ala
                85                  90                  95

Leu Ser Leu Arg Arg Ala Arg Gln Ala Gln Arg Arg Ala Gln Lys Ala
                100                 105                 110

Ser Val Gln Met Thr Gln Glu Arg Lys Glu Leu Val Gln Thr Leu Ile
                115                 120                 125

Gly Ala His Thr Arg His Met Gly Pro Met Phe Asp Gln Phe Val Lys
                130                 135                 140
```

7

```
Leu Arg Pro Pro Ala Tyr Leu Phe Thr His His Arg Pro Ser Ser Pro
145             150             155             160

Leu Val Pro Pro Ala Leu Pro Leu Leu Thr His Phe Ala Asp Val Asn
                165             170             175

Thr Phe Met Val Gln Gln Ile Ile Lys Phe Thr Lys Glu Leu Pro Leu
            180             185             190

Phe Arg Ser Leu Pro Val Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala
        195             200             205

Ala Val Glu Ile Leu His Ile Ser Leu Asn Thr Thr Phe Cys Leu Gln
    210             215             220

Thr Gln Asn Phe Phe Cys Gly Pro Leu Cys Tyr Lys Met Glu Asp Ala
225             230             235             240

Ala His Ala Gly Phe Arg Tyr Glu Tyr Val Glu Leu Ile Phe Arg Phe
            245             250             255

His Gly Thr Leu Lys Arg Leu Gln Leu Gln Glu Pro Glu Tyr Val Leu
            260             265             270

Met Thr Ala Met Ala Leu Phe Ser Pro Asp Arg Pro Gly Ile Thr Gln
        275             280             285

Arg Glu Glu Ile Asp Gln Leu Gln Glu Glu Met Ala Leu Ile Leu Asn
    290             295             300

Asn Tyr Ile Met Glu Gln Gln Pro Arg Pro Gln Ser Arg Phe Leu Tyr
305             310             315             320

Ala Lys Leu Met Gly Leu Leu Ala Glu Leu Arg Ser Ile Asn Asn Ala
            325             330             335

Tyr Ser Tyr Glu Ile Arg Arg Ile Gln Gly Leu Ser Ala Met Met Pro
        340             345             350

Leu Leu Gly Glu Ile Cys Ser
        355
```

<210> 10
<211> 58
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:overlapping
      Oligo 2930

<400> 10
ccataaacgt gttgatatct gcaaagtgtg cgagcagagg caacacgggg ccccgagg   58

<210> 11
<211> 58
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:overlapping
     Oligo 2931

<400> 11
ctctacagcc tccagcctat ctgttcatgc atcaccggcc tttccagcct cggggccc   58

<210> 12
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:boundary of the
     deleted region of the mouse CARbeta gene in CAR
     knock-out construct

<400> 12
cactagaaac catggccagt g   21

<210> 13
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:boundary of the
     deleted region of the mouse CARbeta gene in CAR
     knock-out construct

<400> 13
tgtgtggtgt gggagaccgg gc   22

<210> 14
<211> 201
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:flexible linker

<220>
<221> MOD_RES
<222> (1)..(97)
<223> Gly residues from positions 1-97 may be present or
     absent

<220>
<221> MOD_RES
<222> (105)..(201)
<223> Gly residues from, positions 105-201 may be
     present or absent

<400> 14
Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
 1   5   10   15

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
            20   25   30

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
            35   40   45

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
        50   55   60

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
    65   70   75   80

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
                    85   90   95

Gly Gly Gly Gly Pro Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
            100   105   110

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
        115   120   125

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
        130   135   140

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
    145   150   155   160

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
                165   170   175

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
            180   185   190

Gly Gly Gly Gly Gly Gly Gly Gly Gly
        195   200